SOME WRINKLES WITH FEHLING'S TEST FOR GLUCOSE.

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THESE remarks were mainly inspired by seeing an article a little while ago in one of the journals in which Fehling's test, the test of the multitude for glucose, was quite roughly handled, nor could any good be found in it. It was difficult to make, difficult to keep, annoying to use—just think of the annoyance of mixing two solutions together!—indeed, the only modicum of praise it got was being a good "negative" test. Damned with faint praise, could anything be worse? I purpose to show in what follows that these strictures are in a measure unjustifiable, and that when used in a proper manner, Fehling's solution is a good positive test for glucose in urine, or in anything else, for that matter.

Whatever may be said and written about tests for glucose in urine, and the fallacies and failures of Fehling's test therefor, rest assured that the average practitioner, and sometimes more than the average, usually relies upon it; and for many reasons. It is a reliable negative test used in any old way; this often suffices. It is convenient, easily prepared or gotten from almost any drug store, made in a fairly reliable way. If made of purified crystals of copper sulphate, or even from a good average commercial article, one need only weigh out the requisite amount on even the ordinary prescription balance, and thereby get a pretty accurate solution for quantitative work. At all events, the limit of error of a solution prepared in this manner is usually well within the experimental error of determination, except in a skilled chemist's hands—and for the edification of skilled chemists I am not writing. This advantage of weighing out one salt renders a standardization by means of sucrose into glucose unnecessary unless, of course, very accurate work is to be done, as in some problem investigation or commercial analyses.

Unfortunately Fehling's solution, as ordinarily used for qualitative work, is not in many instances a reliable test; and it is to clear up this difficulty, remove doubts, save time, trouble and annoyance to the general practitioner that I propose a more general use of Fehling's solution in the cold—that's the secret of the whole thing, and it does the work; which is all one ought to expect. One should start out with correct premises, so I give the formulæ of the solutions I have used for many years, and not found wanting; a moderate amount may be mixed ready for use, and kept for perhaps three or four weeks without deterioration.

FOR THE COPPER SOLUTION, A.

Pure sulphuric acid	1.00 gram
Cryst. Copper Sulphate ($CuSO_4 + 5H_2O$)	34.64 "
Water enough to make	500.00 cc

FOR THE ALKALINE SOLUTION, B. (This is where formulæ differ.)

Sodic hydrate sticks, (reasonably pure)	60.00 grams
Sodic-potassic tartrate (recrystalized is best)	175.00 "
Water enough to make	500.00 cc

Equal parts of A and B make Fehling's test solution.

In ordinary testing with this solution a small amount should be boiled and allowed to cool partially before the urine is added, say to a temperature of 180 to 200 degrees Fahrenheit. Then the mixture with urine should not be boiled. It is unnecessary and misleading to boil. If the glucose is there it will soon show by the characteristic yellow or reddish yellow precipitate—seldom red. This alone will give negative results in glucose-free urines that ordinarily would show some reaction when boiled. Better yet, and best, especially where the hot Fehling's has given a slight or an atypical reaction (muddy yellow solution, blue color partially discharged, or other anomalous proceeding), take equal parts of the suspected fluid and Fehling's, cold, mix thoroughly and set aside. Any urine containing one-twentieth of one per cent or more (0.05 per cent) glucose will react, giving a yellow precipitate in the course of twenty-four hours or less, according to the amount of glucose present and the room temperature; I am speaking of an average room temperature of 60 to 65 degrees Fahrenheit. I plead for a more extended use of this simple expedient. Time is not usually precious in these cases. Freedom from doubt is.

I am quite well aware that it may be said that we have the phenylhydrazin test and others for clearing up our doubts. It is true, and practically the phenylhydrazin test is the one oftenest resorted to. In answer to this objection, permit me to remind those who have had experience with phenylhydrazin that it is not all plain sailing, particularly where the percentage of glucose is small. It requires skill and knack and a large amount of practice to get reliable results. Phenylhydrazin hydrochlorate, at least in many samples of Merck's which I have had, has not been particularly good in keeping qualities. The general practitioner is seldom going to use it even if he has it. I am not speaking disparagingly of phenylhydrazin; it is invaluable, accurate, definite and distinguishing (the last by the aid of the microscope and melting point of crystals obtained); I simply do not believe it suitable for the average man.

As regards the limitations of cold Fehling's solution, there are many urines containing neither sugar, glucose nor the ordinary reducing drugs as salicylates, etc., which, with Fehling's as ordinarily used, will give either a typical reaction or one in which we are in doubt; a sluggish reaction, a partial decolorization of the solution and even a slight precipitate of cuprous oxide. This class of urines, I can positively assert, will not in twenty-four hours give a yellow precipitate with equal parts of Fehling's test solution used cold. Anything less or other than a distinct yellow precipitate is not to be counted. These urines usually give a positive reaction when heated with Fehling's solution, from the presence of alloxure bases in excess. Kreatin and kreatinin are favorites for doubtful reactions. How much farther can we go with other sugars or commonly reducing drugs? In answer, I append a little tabular statement giving the commonly occurring reducing agents. Any one can verify this table very easily, as the time consumed in making the tests is practically nil. As is seen, levulose acts exactly similar to dextrose. Any person having levulose in the urine is to all intents and purposes a glycosuric patient. About glycuronic acid: Some people claim this as the chief offender. It is largely so when hot Fehling's is used. It offends no longer with cold; *i. e.*, in reasonable amounts such as will react positively with hot Fehling's always.

I promised you some wrinkles with Fehling's; here is another, and from the chemical standpoint more important: It is indeed a time saver. No originality at all is claimed, only again a plea for a more extended use of it. Perhaps the reason this method is so little used—many chemists seem to be entirely ignorant of it—is because when first reported it was unfortunately reported incorrectly, and gave, when used, very inaccurate results. In 1892, at a meeting of the British Pharmaceutical Conference in Edinburgh, Mr. A. W. Garrard, F. R. S., read a paper on the subject, but had not used his method properly—a mere matter of boiling made the difference between very good and very bad results. The writer soon found this out for himself, and adopted it, and has had no cause for regret since. It is for the quantitative determination of glucose (and, of course, of other reducing agents, if they be present). Take Fehling's

Substance added to Urine. 1030 sp. gr.; acid; no alb.; no sugar.	Grains to 1 ounce	Hours let remain.	A Reaction cold Fehling E. 9 parts	B Reaction hot Fehling's without standing	C Reaction on heating A after standing the specified time	Remarks
Sodii Salicylate	5	36	None	Marked	Slight	
Urine of patient taking 15 grains Soda Salicylate every 4 hours. S. G. 1030; acid; no alb.; no sugar	.....	24	Greenish col. to sol.; no yellow p p t	All blue color discharged; muddy yellow	Same as B, only have to boil longer	
Urine 0.025% alb. (Esbach). S. G. 1035; acid; urates dep.; no casts; Epithelium, much	⅛	24	Greenish col. to sol.; no p p t	M'rk'd yellow discol.; all blue discharged	Same as B	Probably other urinary constituents besides albumin augment this reaction.
Urine, alb., trace; pus, urates, oxalates, yeast cells. S. G. 1032; color dark	.....	24	None	Typical	Typical	Originally a ½% glucose urine; fermented when obtained; phenylhydrazin HCl test fails to give positive reaction.
Urine, 1035 S. G.; very acid; high color; urates dep.; no alb.; no sugar	.....	24	None	Yellow muddy color	Same as B	One of those doubtful urines, just where confusion arises with Fehling's.
Acid Oxalic	5	36	None	None	None	
Maltine	10	1	Typical	Typical	Typical	Reaction in cold very prompt.
Dextrose (glucose)	¼	24	Typical	Typical	Typical	0.050 % solution.
Formalin (40% commercial)	10	24	None	Typical	Typical	
Acetone	10	24	None	None	None	
Manna	5	24	None	Sluggish but typical	None	
Lactose	5	24	None	Typical	Typical	(Milk sugar.)
Alcohol	20	24	None	None	None	
Chloroform, saturated sol. in Urine	.....	24	None	Typical	Slight but typical	
Chloral Hydrate	4	24	Green color to solution	Typical	Muddy yellow color	Chloral 4 grs. to 1 oz. and cold Fehling equal parts let stand 36 hours and then heated fail to give positive reaction.
Levulose	5	24	Typical	Typical	Typical	
Maltose	ℓ	12	Typical	Typical		1% maltose. If Fehling, say 2 cc be heated to boil, taken from flame and 15 drops added and no further heating, reaction very sluggish, beginning in 3–4 min.
Di Acetic Acid	5	24	None	None	None	
Saccharine	3	24	None	None	None	

Column "C" is inserted to show that many reducing agents, in prolonged contact with a strongly alkaline solution containing an oxidizing agent, are more or less decomposed without precipitation of suboxides, thus offering a possible explanation of the efficiency of the method of "Cold Fehling's"

solution (mixed, copper and alkali) 10 cc., dilute with a little water, heat to boiling and add, while boiling, a solution of potassium cyanide (KCN). I use 20 per cent solution or thereabouts. Add drop by drop until the solution is just decolorized while boiling. To this mixture add now 10 cc. Fehling's solution, boil, and while boiling add your glucose solution or urine, best not over 1 per cent solution—add till just decolorized again—(with dark urines the color becomes greenish yellow). This marks the end reaction. Read off on your burette and estimate on the basis of 10 cc. Fehling's only (equals 0.050 grams glucose).

By this method we avoid the tedious waiting for the solution to clear of precipitated cuprous oxide in order to see if the color is all gone; in some estimates with Fehling as usually used it is almost impossible to tell this, thus avoiding two sources of error, the doubt as regards all color gone, and the well-known fact that an excess of hot Fehling's kept in prolonged contact with a deficiency of glucose is reduced more and more as time goes on.

To avoid the troublesome settling of the cuprous oxide in the ordinary analytical operation some one has proposed the addition of a solution of calcic chloride, in small amounts, the lime precipitate carrying down the more flocculent copper oxide. From personal experience I know that the lime precipitate also carries something else with it not so desirable, viz., copper from the solution; hence your glucose percentage reads higher than it should. Various other expedients are adopted, all open to the same objections, time and prolonged contact of Fehling's solution in excess. In fact, if one aims to get accurate results with Fehling's as ordinarily used, he must first make a preliminary determination to know about where he stands. This is obviated by Garrard's method.

I find it convenient to have the KCN in sticks in a tightly closed wide-mouthed bottle. I break off a small piece and pour boiling water over it when I desire to make only an occasional determination, because KCN does not keep well in solution. Ammonia forms and vitiates the results. The whole determination can be carried out in much less time than it takes to tell of it.

In closing, I should like to call attention to a grave error (probably typographical) in one of our most widely used guides to laboratory work, "Clinical Diagnosis," by Simon. This error is contained not only in the earlier editions, but is industriously propagated in later editions; viz., 5th edition 1904, when, in speaking of the standardization of Fehling's by means of sucrose converted into glucose, directions are given to keep at the boiling point for one hour the solution of sugar (cane), with the addition of 22 drops of 0.1 per cent solution of sulphuric acid. Probably a 10 per cent solution of H_2SO_4 is meant, which is not too much. Unless acid to about that amount is added much sucrose remains unconverted at the end of an hour at 100 degrees Centigrade.

The Obstetric Bag.—Dr. John R. Hamilton writes to the *British Medical Journal:* "I believe that the bag of to-day is dangerous; but the danger lies in its size. It can hold too many instruments of offense—more potent to damage than the most subtle germ, and at the same time being the germ's true friend. I would recommend all young practitioners to procure a very small leather bag, if they desire to be successful obstetricians. They will find in the course of twenty-five years few mishaps if they keep their hands clean, and do not fuss too much.".